REMARKS

This submission is in response to the Official Action dated February 5, 2003. Claims 1-62 are pending. Consideration of the above identified application, in view of the above amendment and following remarks, is respectfully requested. No new matter has been added by way of this amendment.

Claim 32 has been amended to recite the contacting of a test enzyme with a substrate and an oxygen donor. This amendment is made to correct for an inadvertent error in the original claims. Claim 39 as filed recites that the oxygen donor of claim 32 is selected from molecular oxygen and a peroxide, but the term oxygen donor had inadvertently been omitted from original claim 32. With this amendment, claim 32 has been amended to recite an oxygen donor, thereby providing proper antecedent basis for the term in claim 39. This amendment is fully supported by the application as filed, e.g., at p. 24, ll. 15-19; p. 35, ll. 8-11; and original claims 1 and 39.

Restriction Requirement

In the Office Action, the Examiner has required election of one of the following groups of claims:

I. Claims 1-31, drawn to a method of detecting an oxidation enzyme, involving the formation of a phenol or a cathecol from a cis-dihydrodiol.

II. Claims 32-56, drawn to a method of detecting an oxidation enzyme, involving the formation of a phenol or a cathecol.

III. Claims 57-62, drawn to a method of detecting an oxidation enzyme involving the formation of a phenol from a phenol ether.

In response, Group I, corresponding to claims 1-31, is hereby elected, **with traverse.** It is respectfully submitted that groups I and II should be rejoined and prosecuted in the same application, for the reasons outlined below.

Groups I and II Should Be Rejoined

The Examiner states that groups I and II set forth patentably distinct processes because group I recites an oxygen donor, which is not required by group II. It is respectfully submitted that an oxygen donor is recited in original claim 39 of group II, thus showing that the method of both groups I and II include an oxygen donor. Furthermore, as amended herewith, claim 32 recites an oxygen donor. Accordingly, there is no distinction between group I and II based on the presence or absence of oxygen donor.

Under 35 U.S.C. §121, "two or more independent and distinct inventions... in one application may... be restricted to one of the inventions". Inventions are "independent" if there is no distinct relationship between the two or more subjects disclosed" (MPEP 802.01). The term "distinct" means that "two or more subjects as disclosed are related... but are capable of separate manufacture, use or sale as claimed, AND ARE PATENTABLE (novel and unobvious) OVER EACH OTHER" (MPEP 802.01) (emphasis in original).

As set forth above, groups I and II are not distinct because of the presence or absence of oxygen donor, since both groups call for the use of oxygen donor. Further, the method of claims 1 and 16 are particular embodiments of claim 32, wherein the product is *cis*-dihydrodiol, and the agent is an acid or cis-dihydrodioldehydrogenase. The recited steps of claims 1, 16 and 32 are otherwise the same. Therefore, in the prior art search, a search for literature regarding a method for detecting an oxygenase enzyme using Gibbs reagent and other features of group I would inevitably turn up any prior art for the method of group II. The Examiner has also classified the subject matter of Groups I and II into the same class (435) and subclasses (18 and 25-28).

Further, as provided by the MPEP, section 806.05(c)II, entitled "SUBCOMBINATION ESSENTIAL TO COMBINATION":

> If there is no evidence that combination AB_{sp} is patentable without the details of B_{sp}, restriction should not be required. Where the relationship between the claims is such that the separately claimed subcombination B_{sp} constitutes the essential distinguishing feature of the combination AB_{sp} as claimed, the inventions are not distinct and a requirement for restriction must not be made, ...

Thus, the subcombination in the instant case, represented by claims 1 and 16 (group I), and the combination, represented by claim 32 (group II), should be prosecuted in the same application.

Accordingly, for all of the above reasons, the claims of Groups I and II, i.e., claims 1-31 and 32-56, should be examined in the same application.

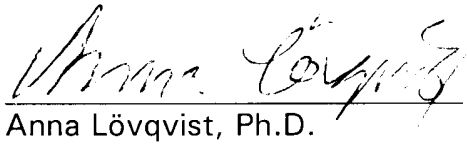
Reconsideration of the restriction requirement as applied to Groups I and II is thus respectfully requested.

Species Election

The Office Action also contains a requirement to elect an enzyme species. In response, toluene dioxygenase is hereby elected as enzyme species. Claims 1, 16, and 32 are generic to the elected species. No claim reads upon the elected species. It is noted that, upon the allowance of a generic claim, Applicant is entitled to consideration of claims to additional, non-elected species.

Early and favorable consideration of this response and the claims is earnestly solicited. If there are any other issues remaining which the Examiner believes could be resolved through either a Supplemental Response or an Examiner's Amendment, the Examiner is respectfully requested to contact the undersigned at the telephone number indicated below.

Respectfully submitted,



Anna Lövqvist, Ph.D.
Limited Recognition Under 37 C.F.R. 10.9(b) (see attached)
Representative of Applicants

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EXPRESS MAIL CERTIFICATE

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L. Stantini A. Stantini

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PATENT TRADEMARK OFFICE

Docket No: 4058/1H222US1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Frances H. ARNOLD et al.

Serial No.: 09/828,599 Art Unit: 1651

Confirmation No.: 5688

Filed: April 5, 2001 Examiner: Sandra E. Saucier

For: SCREENING METHOD FOR THE DISCOVERY AND DIRECTED EVOLUTION OF OXYGENASE ENZYMES

MARK-UP TO RESPONSE TO OFFICIAL ACTION

Hon. Commissioner of
Patents and Trademarks
Washington, DC 20231

March 5, 2003

Sir:

IN THE CLAIMS:

Please amend the claim pursuant to 37 C.F.R. §1.121 as follows:

32. (Amended) A method for detecting an oxidation enzyme comprising the steps of:

a) contacting an test enzyme with a substrate and an oxygen donor to promote the formation of a product from the substrate;

b) contacting the product with an agent to promote the formation of a modified product, wherein the modified product is selected from the group consisting of a phenol and a catechol;

c) contacting the modified product with 2,6-dichloroquinone-4-chloroimide (Gibbs reagent) to promote the formation of a detectable composition; and

d) testing for the detectable composition, wherein the presence of the detectable composition indicates that the test enzyme is an oxidation enzyme.

EXPRESS MAIL CERTIFICATE

Date 3/5/03 Label No. EV 294036894 US

I hereby certify that, on the date indicated above, this paper or fee was deposited with the U.S. Postal Service & that it was addressed for delivery to the Assistant Commissioner for Patents, Washington, DC 20231 by "Express Mail Post Office to Addressee" service.

A. Stantini A. Stantini
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For: SCREENING METHOD FOR THE DISCOVERY AND DIRECTED EVOLUTION OF OXYGENASE ENZYMES

EXAMINER'S COURTESY COPY OF PENDING CLAIMS

Hon. Commissioner of
Patents and Trademarks
Washington, DC 20231

March 5, 2003

Sir:

1. A method for detecting an oxidation enzyme comprising the steps of:

(a) contacting a test enzyme with a substrate and an oxygen donor to promote the formation of a cis-dihydrodiol from the substrate and the oxygen donor;

(b) subjecting the cis-hydrodiol to acidic conditions to promote the formation of a phenol from the cis-dihydrodiol;

(c) contacting the phenol with 2,6-dichloroquinone-4-chloroimide (Gibbs reagent) under neutral conditions to promote the formation of a detectable composition; and

(d) testing for the detectable composition, wherein the presence of the detectable composition indicates that the test enzyme is an oxidation enzyme.

2. The method of claim 1, wherein the oxidation enzyme is selected from the group consisting of a monooxygenase enzyme and a dioxygenase enzyme.

3. The method of claim 1, wherein the oxidation enzyme is selected from the group consisting of toluene dioxygenase, biphenyl dioxygenase, naphthalene dioxygenase, methane monooxygenase, chloroperoxidase, cytochrome P450, phenol hydroxylase, dehalogenase, and microperoxidase.

4. The method of claim 1, wherein the test enzyme is a mutant enzyme or a wild-type enzyme.

5. The method of claim 1, wherein the substrate is selected from the group consisting of an aromatic hydrocarbon and a halogenated ethylene.

6. The method of claim 1, wherein the substrate is selected from benzene, toluene, t-butylbenzene, 1,2,4-trimethylbenzene, flurorbenzene, chlorobenzene, bromobenzene, iodobenzene, benzoic acid, p-methoxybenzoic acid, 2-napthoic acid, benzamide, pyridine, and 4-picoline.

7. The method of claim 1, wherein the oxygen donor is selected from the group consisting of molecular oxygen and a peroxide.

8. The method of claim 1, wherein the acidic conditions are provided by lowering the pH to about 2.5.

9. The method of claim 1, wherein the neutral conditions are provided by increasing the pH to within the range from about 7 to about 9.

10. The method of claim 1, wherein the test enzyme is expressed in a host cell, and the substrate and oxygen donor contacted with the host cell.

11. The method of claim 10, wherein the host cell comprises a plasmid comprising a gene encoding the test enzyme.

12. The method of claim 10, wherein the host cell is attached to a solid support.

13. The method of claim 12, wherein the solid support is selected from the group consisting of agar and a membrane.

14. The method of claim 12, wherein colonies of multiple host cells are spread on the solid support.

15. The method of claim 1, wherein the detectable composition is a colored product detectable by visual inspection, spectrometry, or digital imaging.

16. A method for detecting an oxidation enzyme comprising the steps of:

(a) contacting an test enzyme with a substrate to promote the formation of a cis-dihydrodiol from the substrate;

(b) contacting the cis-hydrodiol with cis-dihydrodiol dehydrogenase to promote the formation of a catechole from the cis-hydrodiol;

(c) contacting the catechol with 2,6-dichloroquinone-4-chloroimide (Gibbs reagent) to promote the formation of a detectable composition; and

(d) testing for the detectable composition, wherein the presence of the detectable composition indicates that the test enzyme is an oxidation enzyme.

17. The method of claim 16, wherein the oxidation enzyme is selected from the group consisting of a monooxygenase enzyme and a dioxygenase enzyme.

18. The method of claim 16, wherein the oxidation enzyme is selected from the group consisting of toluene dioxygenase, biphenyl dioxygenase, naphthalene dioxygenase, methane monooxygenase, chloroperoxidase, cytochrome P450, phenol hydroxylase, dehalogenase, and microperoxidase.

19. The method of claim 16, wherein the test enzyme is a mutant enzyme or a wild-type enzyme.

20. The method of claim 16, wherein the substrate is selected from the group consisting of an aromatic hydrocarbon and a halogenated ethylene.

21. The method of claim 16, wherein the substrate is selected from benzene, toluene, t-butylbenzene, 1,2,4-trimethylbenzene, flurorbenzene, chlorobenzene, bromobenzene, iodobenzene, benzoic acid, p-methoxybenzoic acid, 2-napthoic acid, benzamide, pyridine, and 4-picoline

22. The method of claim 16, wherein the oxygen donor is selected from the group consisting of molecular oxygen and a peroxide.

23. The method of claim 16, wherein the test enzyme is expressed in a host cell, and the substrate and oxygen donor are contacted with the host cell.

24. The method of claim 23, wherein the host cell also expresses cis-dihydrodiol dehydrogenase.

25. The method of claim 24, wherein the host cell comprises a plasmid comprising genes encoding the test enzyme and cis-dihydrodiol dehydrogenase.

26. The method of claim 23, wherein the host cell is attached to a solid support.

27. The method of claim 26, wherein the solid support is selected from the group consisting of agar and a membrane.

28. The method of claim 26, wherein colonies of multiple host cells are spread on the solid support.

29. The method of claim 16, further comprising contacting cis-dihydrodiol dehydrogenase with a coenzyme.

30. The method of claim 28, wherein the coenzyme is NAD+.

31. The method of claim 16, wherein the detectable composition is a colored product detectable by visual inspection, spectrometry, or digital imaging.

32. (Amended) A method for detecting an oxidation enzyme comprising the steps of:

a) contacting an test enzyme with a substrate and an oxygen donor to promote the formation of a product from the substrate;

b) contacting the product with an agent to promote the formation of a modified product, wherein the modified product is selected from the group consisting of a phenol and a catechol;

c) contacting the modified product with 2,6-dichloroquinone-4-chloroimide (Gibbs reagent) to promote the formation of a detectable composition; and

d) testing for the detectable composition, wherein the presence of the detectable composition indicates that the test enzyme is an oxidation enzyme.

33. The method of claim 32, wherein the oxidation enzyme is selected from the group consisting of a monooxygenase enzyme and a dioxygenase enzyme.

34. The method of claim 32, wherein the oxidation enzyme is selected from the group consisting of toluene dioxygenase, biphenyl dioxygenase, naphthalene dioxygenase, methane monooxygenase, chloroperoxidase, cytochrome P450, phenol hydroxylase, dehalogenase, and microperoxidase.

35. The method of claim 32, wherein the test enzyme is a mutant enzyme or a wild-type enzyme.

36. The method of claim 32, wherein the test enzyme is expressed in a host cell, and the substrate and oxygen donor are contacted with the host cell.

37. The method of claim 32, wherein the substrate is selected from the group consisting of an aromatic hydrocarbon and a halogenated ethylene.

38. The method of claim 32, wherein the substrate is selected from benzene, toluene, t-butylbenzene, 1,2,4-trimethylbenzene, flurorbenzene, chlorobenzene, bromobenzene, iodobenzene, benzoic acid, p-methoxybenzoic acid, 2-napthoic acid, benzamide, pyridine, and 4-picoline

39. The method of claim 32, wherein the oxygen donor is selected from the group consisting of molecular oxygen and a peroxide.

40. The method of claim 32, wherein the product is selected from a cis-dihydridiol, an alkylated benzene, a halogenated benzene, and a carboxylated benzene.

41. The method of claim 40, wherein the product is anthranilic acid

42. The method of claim 32, wherein the agent is an acid.

43. The method of claim 32, wherein the agent is an enzyme.

44. The method of claim 42, wherein the enzyme is a second oxidation enzyme.

45. The method of claim 43, wherein the product is a cis-dihydrodiol and the second oxidation enzyme is a cis-dihydrodiol dehydrogenase.

46. The method of claim 43, wherein the product is a halogenated benzene, and the second oxidation enzyme is a dehalogenase.

47. The method of claim 43, wherein the product is selected from the group consisting of alkylated and carboxylated benzene, an the second oxidation enzyme is selected from the group consisting of cytochrome P450 and a peroxidase.

48. The method of claim 43, wherein the product is anthranilic acid, and the second oxidation enzyme is anthranilite monooxygenase.

49. The method of claim 36, wherein the host cell also expresses a second oxidation enzyme, which second oxidation enzyme promotes the formation of a modified product.

50. The method of claim 49, wherein the host cell comprises a plasmid comprising genes encoding the test enzyme and the second oxidation enzyme.

51. The method of claim 49, wherein the product is a cis-dihydrodiol and the second oxidation enzyme is a cis-dihydrodiol dehydrogenase.

52. The method of claim 51, further comprising contacting the cis-dehydydiol dehydrogenase with NAD+.

53. The method of claim 36, wherein the host cell is attached to a solid support.

54. The method of claim 53, wherein the solid support is selected from the group consisting of agar and a membrane.

55. The method of claim 53, wherein colonies of multiple host cells are spread on the solid support.

56. The method of claim 32, wherein the detectable composition is a colored product detectable by visual inspection, spectrometry, or digital imaging.

57. A method for detecting an oxidation enzyme comprising the steps of:

(a) contacting test enzyme with a substrate to promote the formation of a phenol ether, wherein the hydroxyl-group is attached to the aromatic part of the phenol ether;

(b) contacting the phenol ether with 2,6-dichloroquinone-4-chloroimide (Gibbs reagent) to promote the formation of a detectable composition; and

(c) testing for the detectable composition, wherein the presence of the detectable composition indicates that the test enzyme is an oxidation enzyme.

58. The method of claim 57, wherein the oxidation enzyme is cytochrome P450.

59. The method of claim 57, wherein the test enzyme is a mutant enzyme or a wild-type enzyme.

60. A method for detecting an oxidation enzyme comprising the steps of:

(a) contacting a test enzyme with a substrate to promote the formation of a phenol ether, wherein the hydroxyl-group is attached to the ether part of the phenol ether;

(b) allowing the phenol ether to dissociate into an aldehyde and a phenol;

(c) contacting the phenol with 2,6-dichloroquinone-4-chloroimide (Gibbs reagent) to promote the formation of a detectable composition; and

(d) testing for the detectable composition, wherein the presence of the detectable composition indicates that the test enzyme is an oxidation enzyme.

61. The method of claim 60, wherein the oxidation enzyme is cytochrome P450.

62. The method of claim 60, wherein the test enzyme is a mutant enzyme or a wild-type enzyme.

BEFORE THE OFFICE OF ENROLLMENT AND DISCIPLINE
UNITED STATES PATENT AND TRADEMARK OFFICE

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This document constitutes proof of such recognition. The original of this document is on file in the Office of Enrollment and Discipline of the United States Patent and Trademark Office.

Expires: February 4, 2004

Harry I. Moatz
Director of Enrollment and Discipline